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### Spectral analysis of blood stains at the crime scene

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## **6 - VISUALIZATION OF LATENT BLOOD STAINS USING VISIBLE REFLECTANCE HYPERSPECTRAL IMAGING AND CHEMOMETRICS**

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The detection of latent traces is an important aspect of crime scene investigations. Blood stains on black backgrounds can be visualized using chemiluminescence, which is invasive and requires a darkened room, or near infrared photography, for which investigators need to change filters manually to optimize contrast. We demonstrated the performance of visible reflectance hyperspectral imaging (400-720 nm) for this purpose. Several processing methods were evaluated: single wavelength bands, ratio images, Principal Component Analysis (PCA) and “SIMPLe-to-use Interactive Self-modeling Mixture Analysis” (SIMPLISMA). Using these methods, we were able to enhance the contrast between blood stains and 12 different fabrics. On black cotton, blood dilutions were visible with a minimal concentration of 25% of whole blood. The hyperspectral camera system used in this study is portable and wireless, which makes it suitable for crime scene use. The described technique is non-contact and non-destructive, so all traces are preserved for further analysis.

## 6.1. INTRODUCTION

The visualization of latent blood stains is of paramount importance for crime scene investigators. Because the human ability to visually detect traces is limited, various techniques are developed which enhance the contrast between blood stains and their backgrounds, using the intrinsic properties of blood stains or exogenous dyes. Several chemical reagents can be sprayed onto the blood stains to induce luminescence, e.g. luminol, bluestar or fluorescein<sup>110</sup>. Drawbacks of these methods are that a completely dark environment is needed to visualize the luminescence and that photographs have to be taken quickly. For the visualization of blood stains on dark backgrounds, the feasibility to use both analogue<sup>111, 112</sup> and digital<sup>113</sup> near infrared photography were successfully explored, both involving the use of several filters which have to be changed manually.

To overcome this problem, Schuler, Kish and Plese<sup>114</sup> recently introduced near infrared hyperspectral imaging for the non-destructive detection of blood stains on dark backgrounds. Hyperspectral cameras automatically record the back scattered light of many narrow wavelength bands, resulting in a data set with monochrome images for each wavelength. They demonstrated that the contrast between blood stains and 3 black fabrics could be enhanced using hyperspectral imaging systems operating in the wavelength ranges from 650-1100 nm and from 960-1650 nm. We explored the performance of a visible reflectance hyperspectral imaging system operating in the wavelength range 400-720 nm to visualize blood stains on 12 different black fabrics. We developed a low-cost, portable and wireless systems, which can be transported to the crime scene to record large samples or even the entire crime scene.

Using hyperspectral imaging latent blood stains can be distinguished from their background based on spectral differences. Several processing methods can be used to increase the contrast between the blood stains and backgrounds. The easiest way to search for latent blood stains is by viewing all separate images individually or by selecting one wavelength based on prior knowledge of absorption spectra of the blood stain *and* its background. To

enhance spectral differences that are difficult to detect in individual images, a ratio of two wavelengths can be calculated. Ratio images may suppress the effect of variable illumination and topographic variations and may provide information not available in any of the single wavelengths. To make use of all subtle information present in the dataset, advanced chemometrical techniques can be used, e.g. Principal Component Analysis (PCA)<sup>115</sup> or “SIMPLISMA-to-use Interactive Self-modeling Mixture Analysis” (SIMPLISMA)<sup>116</sup>. PCA converts the original wavelength bands into new variables, called principal components (PC), which are linear combinations of the original wavelength bands<sup>115</sup>. Each new PC will account for most of the variance in the observed wavelengths. Because blood stains and their backgrounds are spectrally different, it is expected that the first two PCs will emphasize the contrast. The SIMPLISMA algorithm searches pixels with a spectral contribution from one pure component, which are assumed to be present in the hyperspectral datacube<sup>116</sup>. Division of the hypercube by the pure spectra of the separate components gives the concentration profiles of these components. In a hyperspectral image showing a blood stain on a substrate, the concentration profiles of the first two pure components are expected to show the distribution of the blood and the substrate respectively. In this chapter, the above described processing methods are evaluated and the contrast in the resulting images is compared.

## **6.2. MATERIALS AND METHOD**

### **6.2.a SAMPLES**

For this study, three sample sets were created:

Fabrics) 12 different black fabrics with blood stains. The materials of the fabrics are listed in Table 6.1. All fabrics used were cut from second hand clothing, worn and washed regularly prior to application of the blood. The size of the samples was approximately 2 cm by 2 cm. On each fabric, one drop of blood was deposited directly from the fingertip.

Blood dilutions) A dilution series of freshly drawn blood stains in water, of which 1 ml was deposited on black cotton. Concentrations used were 100%, 50%, 25%, 12.5% and 0% of blood.

T-shirt) A cotton T-shirt with a spatter pattern of whole blood and several random blood dilutions.

All blood stains were left to dry and aged for one week prior to the hyperspectral imaging.

Table 6.1. List of materials of the 12 different fabrics.

<b>Sample</b>	<b>Material</b>	<b>Sample</b>	<b>Material</b>
<b>1</b>	100% polyester	<b>7</b>	90% polyamide, 10% elasthan
<b>2</b>	85% polyamide, 15% elasthan	<b>8</b>	65% polyester, 32% viscose, 3% elasthan
<b>3</b>	100% Merino wool	<b>9</b>	92% nylon, 8% elasthan
<b>4</b>	100% cotton	<b>10</b>	70% acryl, 30% wool
<b>5</b>	97% cotton, 3% lycra	<b>11</b>	100% polyester
<b>6</b>	100% nylon	<b>12</b>	55% polyester, 45% new wool

## 6.2.b HYPERSPECTRAL IMAGING

Hyperspectral images of all samples were recorded using a combination of a monochromatic CCD camera (Pixelfly vga; PCO; Kelheim, Germany), a 50 mm c-mount lens, and a liquid crystal tuneable filter (VariSpec VIS, 7 nanometre resolution; CRi; Woburn, USA) in the wavelength range from 400 to 720 nm, with a step size of 1 nm. The exposure time per wavelength was set to 40 ms, which resulted in a total scanning time of less than 15 seconds. All components are built into a wireless hyperspectral imaging system with a built-in acquisition board and computer (see Figure 6.1). Exposure time, focus, and wavelength range, were adjusted on the touch screen of the system. An in

house developed ring of white, blue and cyan LED's and an external halogen light source were used for illumination.



Figure 6.1. Custom made wireless hyperspectral imaging system, consisting of a liquid crystal tuneable filter, a CCD camera, a lens, a built-in acquisition board, computer, a battery, and a touch screen. A single wavelength band is shown on the live view of the camera.

Hyperspectral images are analogous to a stack of images, each acquired at a narrow spectral band. The resulting data set is a three-dimensional block of data, the so-called hypercube described in **Chapter 2**, with two spatial ( $x,y$ ) dimensions and one wavelength ( $\lambda$ ) dimension<sup>117</sup>. This hypercube provides images for each wavelength ( $\lambda_i$ ) and a spectrum can be obtained from each individual pixel ( $x_i,y_i$ ), as depicted in Figure 6.2.

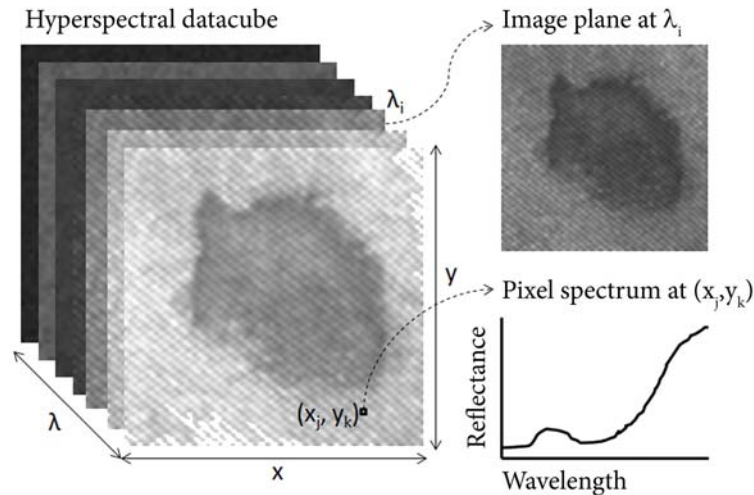


Figure 6.2. Hypercube of a blood stain on black fabric, with two spatial ( $x,y$ ) and one wavelength ( $\lambda$ ) dimension. From the hypercube an image plane is shown for one wavelength ( $\lambda_i$ ) and a spectrum is obtained from one pixel ( $x_j,y_k$ ).

### 6.2.c DATA ANALYSIS

Four different processing methods were used for the visualization of the blood stains on black fabrics and compared based on Fisher's ratio, a measure for the discriminating power between two groups<sup>118</sup>. To calculate Fisher's ratio, a region of interest containing the blood stain was selected manually. Less concentrated parts of the blood stain were included in this region. For each blood stain we compared four data analysis methods, based on the same regions of interest, as described below. The number of pixels used differed from stain to stain.

Band) A single wavelength band was chosen from the datacube. To visually find the wavelength with the highest contrast between the blood stain and the background, each wavelength can be viewed separately. However, as the amount of wavelengths is high in hyperspectral images (321 in our study) this method is time consuming. To reduce the amount of work and to increase the objectivity, we decided to use Fisher's ratio to find the optimal wavelength, which is only possible if the location of the blood stain is known a priori.

Ratio) A ratio image was calculated between two wavelength bands. When  $n$  is the total amount of wavelengths there are  $(n^2-n)/2$  unique combinations of wavelengths, which leads to a substantial amount of possible ratios (51360 in our study). Again, Fisher's ratio was used to select the optimal combination of wavelengths to distinguish the blood stain from its background.

PCA) A scores image of one principle component (PC) was chosen. Although the number of PCs can maximally reach the number of wavelengths  $n$  (321 in this study), PCA is defined such that the first PC describes the highest possible variance in the data and each succeeding component in turn describes the highest variance possible. For a hyperspectral image of a blood stain and a background, the highest variance is expected to result from the different chemical compositions of the two components. As a consequence, the first PCs are expected to show this variance. Taking into account that inhomogeneous lighting conditions or textures can also cause some variance, we expect that the first 4 PCs are sufficient for the visualization of blood stains. Fisher's ratio was again used to select the optimal PC.

SIMPLISMA) A concentration distribution image of one pure component was chosen. This pure spectrum was based on Windig's discovery that the ratio of the standard deviation to the mean for the same variable correlates to the purity of the variable<sup>116</sup>. Because the goal was to create contrast between blood and the background, the algorithm was used to find 2 pure spectra and the corresponding concentration images. From these 2 images, the best one was chosen based on Fisher's ratio.

All results are depicted in grayscale images, with data stretching over the dynamic range. As a result of the different processing methods used, blood stains were sometimes shown as a dark stain on a light background, in other cases as a light stain on a dark background. For the ease of visual comparison, all images showing a light stain on a dark background were inverted. No further image processing or contrast enhancement was applied.

All data analysis was performed using custom-made scripts written in MATLAB (The Mathworks Inc., Natick, Massachusetts, USA). The SIMPLISMA algorithm was made available by Jaumot et al<sup>119</sup>.



### 6.3. RESULTS

Fabrics) White light photographs of all samples on the 12 different fabrics are shown in Figure 6.3. Although the blood stains are visible on some of the fabrics, the contrast is poor. Figure 6.3 also shows the results of hyperspectral imaging with the different processing methods. All these methods improved the contrast between the blood stains and the background.

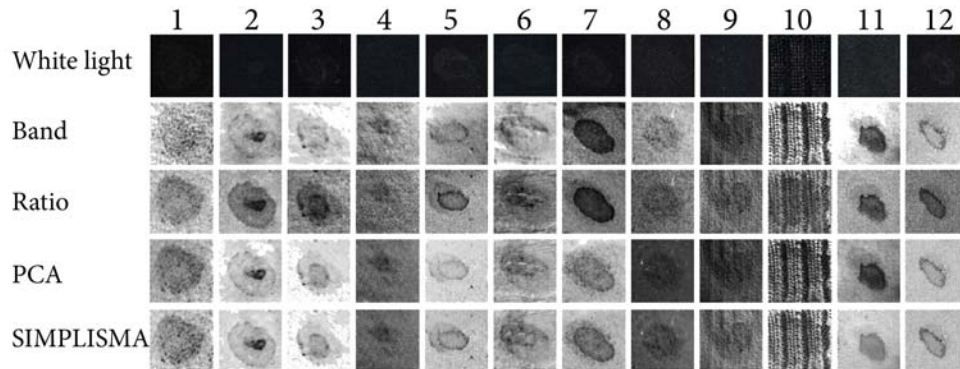


Figure 6.3. White light photographs of all samples on the 12 different fabrics and the results of the different hyperspectral imaging methods: band images, ratio images, PCA images and SIMPLISMA images.

Table 6.2. Fisher's ratios calculated for the different methods (band, ratio, PCA, SIMPLISMA) and the different fabrics (1-12). The highest value for each fabric is printed in bold script.

	1	2	3	4	5	6	7	8	9	10	11	12
<b>Band</b>	0.22	0.59	1.44	<b>1.01</b>	0.45	0.68	1.65	0.72	0.24	0.21	4.38	0.86
<b>Ratio</b>	2.49	<b>2.68</b>	1.91	0.47	1.67	1.02	<b>8.03</b>	0.39	<b>0.36</b>	<b>0.30</b>	<b>5.05</b>	<b>2.31</b>
<b>PCA</b>	<b>2.76</b>	0.95	1.74	0.81	1.38	1.28	2.79	0.47	0.23	0.17	3.73	1.65
<b>SIMPLISMA</b>	1.78	1.23	<b>1.92</b>	0.68	<b>1.92</b>	<b>1.47</b>	5.30	<b>1.00</b>	0.32	0.24	3.27	<b>2.31</b>

Table 6.2 lists Fisher's ratios for each method and each fabric, which is an objective measure to compare the contrast between the blood stains and the backgrounds. This shows that ratio images gave the best result for 6 fabrics,

SIMPLISMA for 5 fabrics and both single wavelength bands and PCA for 1 fabric.

Table 6.3 shows which wavelength, which combination of wavelengths, which principal component, or which concentration image gave the best result for the different methods respectively. The results show that for both the single wavelength band images and the ratio images the chosen wavelengths highly depend on the fabric. Even for similar materials (4-5, 2-7, and 1-11) the wavelengths are different, which implies that the dyes used for blackening are of influence. When PCA was used, PC 2 shows the best contrast in 9 cases, and PC 1 in the other 3 cases, which demonstrates that it is sufficient to use only 2 PC's. The same results were found when SIMPLISMA was used. In that case, we only calculated the concentration images of 2 pure spectra.

Table 6.3. Selected wavelength bands or components resulting in the highest Fisher ratios for the different methods (band, ratio, PCA, SIMPLISMA) and the different fabrics (1-12).

	1	2	3	4	5	6	7	8	9	10	11	12
<b>Band</b>	666	597	611	590	597	610	720	610	719	720	718	598
<b>Ratio</b>	678	646	416	424	665	634	674	411	574	607	662	663
	720	686	636	612	689	720	720	635	720	720	691	703
<b>PCA</b>	2	2	2	2	2	2	2	2	1	1	1	2
<b>SIMPLISMA</b>	2	2	2	2	2	2	2	2	1	1	1	2

Dilutions) Figure 6.4 shows white light photographs and the results of the different hyperspectral image analysis methods for all blood dilutions. Table 6.4 lists the corresponding Fisher ratios. Although the Fisher ratio increased with concentration, blood stains with a minimal concentration of 25% could be observed. For all these blood stains, SIMPLISMA had the best performance.

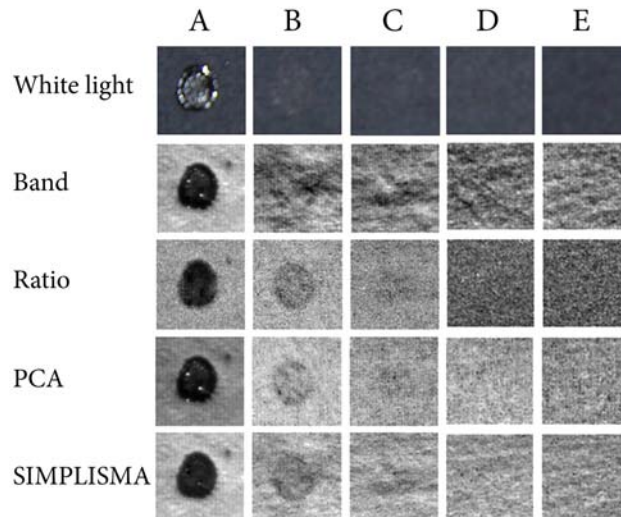


Figure 6.4. White light photographs of all blood dilutions (A: 100%, B: 50%, C: 25%, D: 12.5%, E: 0%) and the results of the different hyperspectral imaging methods: band images, ratio images, PCA images and SIMPLISMA images.

Table 6.4. Fisher ratios calculated for the different methods (band, ratio, PCA, SIMPLISMA) and the different blood dilutions (A-E). The highest value for each fabric is printed in bold script. Because no contrast was observed in dilution D and E, no Fisher ratio could be calculated.

	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>
<b>Band</b>	12.21	0.26	0.05		
<b>Ratio</b>	8.11	0.85	0.10		
<b>PCA</b>	9.15	1.15	0.06		
<b>SIMPLISMA</b>	<b>16.74</b>	<b>1.42</b>	<b>0.13</b>		

T-shirt) A white light photograph of the black cotton T-shirt with diluted and pure blood stains is shown in Figure 6.5. Although several blood stains are visible, the contrast with the background is poor. Figure 6.5 demonstrates that this contrast can be enhanced using hyperspectral imaging and several processing methods. No Fisher ratios were calculated, because this requires selection of all blood stains. Optimal wavelengths for band and ratio images were chosen based on the results of the dilution series. Because both

PCA and SIMPLISMA will be disturbed when more than two components are present, prior to analysis the T-shirt was selected manually to separate it from the environment.

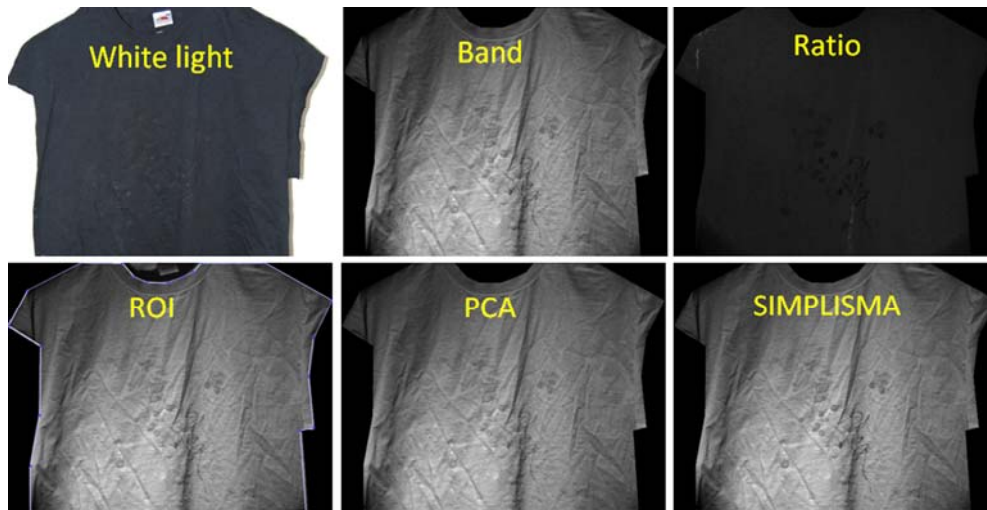


Figure 6.5. White light photograph of black cotton T-shirt with diluted and pure blood stains, the selected region of interest (ROI) and the resulting band, ratio, PCA and SIMPLISMA images. In the ratio image, the contrast was enhanced manually by adjusting the scale of the colour bar.

Visually, the resulting band, PCA and SIMPLISMA images are similar. The ratio image created with an automatic dynamic range showed several white pixels with an infinite pixel value, caused by black pixels in the original wavelength band through which was divided. Because these infinite pixel values influenced the automatic scaling of the greyscale values, the scale of the colourbar was manually adapted. After this manual scaling, a very high contrast between the blood stains and the T-shirt was observed (Figure 6.5). Most features caused by shadows and folds disappeared in this image.

## 6.4. DISCUSSION AND CONCLUSION

We demonstrated the capability of a visible reflectance hyperspectral imaging system (400-720 nm) to visualize blood stains on black backgrounds. Using this system, combined with several processing methods, we were able to enhance the contrast between blood stains and 12 different fabrics. On black cotton, blood dilutions were visible with a minimal concentration of 25%. The custom-made hyperspectral imaging system used in this study is portable and wireless, can be transported to the crime scene to record large samples or even the entire crime scene. Because this technique is non-contact and non-destructive, all traces are preserved for further analysis.

The four processing methods evaluated in this study all enhance the contrast between blood stains and black backgrounds compared to white light photography. Single wavelength bands are easily depicted in the live view of the camera, because no processing is needed. Using this live view, investigators can search for latent blood stains at the crime scene. The optimal wavelength depends on the spectral properties of the blood stains and the background. There may also be a variability between different stains on the same background, which was not tested in this study. Prior knowledge about the absorption properties can be used to choose a wavelength, or a full wavelength sweep can be viewed. Ratio images highly improve the contrast, as texture and lighting inhomogeneities are reduced. However, there are many possible combinations of wavelengths to create ratio images. To find the optimal combination, knowledge about the location of the blood stain is needed. PCA and SIMPLISMA are both unsupervised processing methods, which make more use of the spectral data. Although SIMPLISMA outperforms PCA, it is computationally intensive.

In their preliminary observations Schuler et al showed similar results using two hyperspectral imaging systems which perform in the near infrared wavelength range (650-1100 nm and 950-1650 nm). Although the absorption of blood is higher in the visible range than in the near infrared<sup>1, 19, 82</sup>, on several backgrounds it may be impossible to find a contrast in the visible wavelength range, motivating the use of more expensive near infrared hyperspectral

imaging systems. Apart from blood stains, other latent traces may become visible using hyperspectral imaging and the processing methods described. It may be possible to discriminate blood stains from other substances based on their absorption properties, but this identification task is expected to be hampered by dark backgrounds, because of the dominant light absorption of the background. Instead of the visible wavelength range, near infrared wavelengths can be used for this task (**Chapter 7**).

In conclusion, visible hyperspectral imaging is useful for the visualization of blood stains on black backgrounds. Several processing methods enhance the contrast compared to white light photographs. To search for latent blood stains, a single wavelength band can be shown on the live view of the camera. Afterwards, ratio images, PCA or SIMPLISMA can be used to enhance the contrast.